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Report

Optogenetic Activation of Dorsal Raphe Serotonin Neurons Enhances Patience for Future Rewards

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Summary

Serotonin is a neuromodulator that is involved extensively in behavioral, affective, and cognitive functions in the brain. Previous recording studies of the midbrain dorsal raphe nucleus (DRN) revealed that the activation of putative serotonin neurons correlates with the levels of behavioral arousal [1], rhythmic motor outputs [2], salient sensory stimuli [3–6], reward, and conditioned cues [5–8]. The classic theory on serotonin states that it opposes dopamine and inhibits behaviors when aversive events are predicted [9–14]. However, the therapeutic effects of serotonin signal-enhancing medications have been difficult to reconcile with this theory [15, 16]. In contrast, a more recent theory states that serotonin facilitates long-term optimal behaviors and suppresses impulsive behaviors [17–21]. To test these theories, we developed optogenetic mice that selectively express channelrhodopsin in serotonin neurons and tested how the activation of serotonergic neurons in the DRN affects animal behavior during a delayed reward task. The activation of serotonin neurons reduced the premature cessation of waiting for conditioned cues and food rewards. In reward omission trials, serotonin neuron stimulation prolonged the time animals spent waiting. This effect was observed specifically when the animal was engaged in deciding whether to keep waiting and was not due to motor inhibition. Control experiments showed that the prolonged waiting times observed with optogenetic stimulation were not due to behavioral inhibition or the reinforcing effects of serotonergic activation. These results show, for the first time, that the timed activation of serotonin neurons during waiting promotes animals' patience to wait for a delayed reward.

Results

We used optogenetic methods to control serotonergic neuronal activity with precise timing [22, 23]. We created transgenic mice that expressed the channelrhodopsin 2 variant ChR2(C128S) in serotonin neurons under the control of the tryptophan hydroxylase 2 (Tph2) promoter, which was enhanced with the tetracycline-controlled transcriptional activator (tTA)::tTA-dependent promoter (tetO) system [24, 25]. The selective expression of ChR2 fused to enhanced yellow fluorescent protein (ChR2-EYFP) in the serotonin neurons in the dorsal raphe nucleus (DRN) was verified via immunohistochemical staining for Tph2 and EYFP (Figure 1A). Among the Tph2-positive cells (Tph2-immunoreactive [IR], n = 284 from 5 brain sections of 5 mice), 81.9% ± 4.5% (mean ± SEM) of the cells were colabeled with EYFP staining (EYFP-positive cells, IR). All EYFP-positive cells (n = 229) were Tph2-positive cells, indicating that the ChR2 was expressed only in the serotonin neurons.

Current clamp experiments with variable strengths of blue light showed that the stimulation of 0.01 mW was sufficient to activate the excitatory current (Figures 1B and 1C). In vitro intracellular recording confirmed that a short pulse of blue light (1.3 mW, 500 ms) caused vigorous sustained spiking (baseline firing rate [pre], 1.50 ± 0.15 Hz; during optogenetic activation [blue], 5.66 ± 0.25 Hz; mean ± SEM; n = 10), which was stopped by yellow light stimulation (1.1 mW, 500 ms) with a slight inhibition of firing (from yellow light onset to 5 s afterward [post-1], 0.66 ± 0.23 Hz; from 5 s after yellow light onset to 10 s after yellow light onset [post-2], 1.00 ± 0.30 Hz) ($F(3,27) = 98.04$, $p < 10^{-6}$, one-way repeated measures ANOVA; $p = 7.0 \times 10^{-6}$ for pre versus blue, $p = 0.0014$ for pre versus post-1, $p = 0.14$ for pre versus post-2, post hoc Bonferroni test) (Figures 1D and 1E). We examined whether yellow light stimulation inhibited serotonin neural activity via in vitro electrophysiological recordings. 500 ms of yellow light simulation did not change the 5 s pre- and postfiring rate ($t(9) = 0.12$, $p = 0.23$, n = 10, paired t test) (Figure 1F). We found that 5 s of yellow light stimulation had no significant effect on the population (baseline firing rate, 2.66 ± 0.13 Hz; during yellow light, 2.52 ± 0.18 Hz; after yellow light, 2.72 ± 0.12 Hz; n = 10; $F(2,18) = 2.36$, $p = 0.14$, one-way repeated measures ANOVA) (Figures 1G and 1H).

To confirm the effectiveness of the optogenetic stimulation, we performed an in vivo microdialysis experiment in four mice, with an optical fiber implanted above the DRN and a microdialysis probe in the medial prefrontal cortex (Figure 1I; see Figure S1, available online, for the implant locations). Although the continuous yellow light stimulation had no significant effect on serotonin efflux, both the continuous and the 1 s transient blue light stimulations caused a robust increase in serotonin release (103% ± 5% of the baseline for continuous yellow light, n = 7; 174% ± 9% for continuous blue light, n = 8; 167% ± 18% for 1 s of transient blue light, n = 6) ($t(6) = 0.62$, $p = 0.56$ compared with the baseline for continuous yellow light; $t(7) = 6.94$, $p = 2.24 \times 10^{-4}$ for continuous blue light; $t(5) = 3.79$, $p = 0.012$ for 1 s of transient blue light; paired t test) (Figure 1J).

Optogenetic Stimulation of Serotonin Neurons Reduced Tone Wait Errors

Mice (n = 5) were trained to perform a sequential tone-food waiting task that required them to wait for a delayed tone

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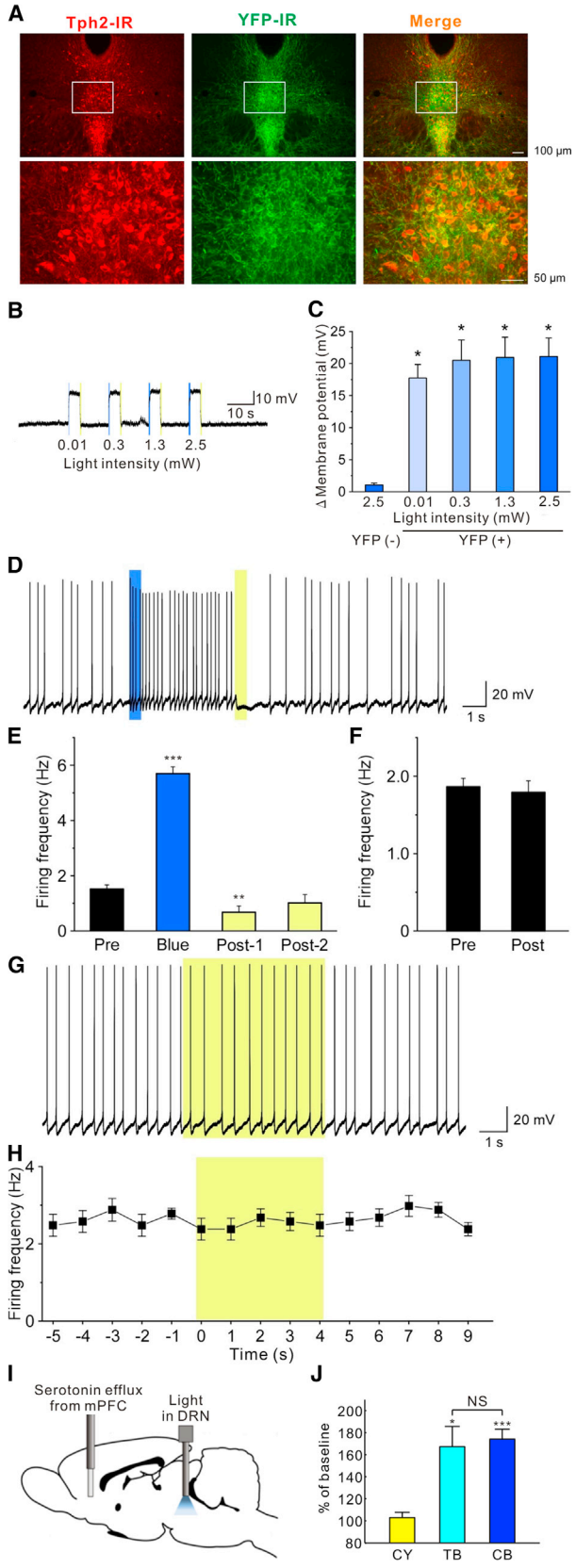

Figure 1. ChR2(C128S) Is Specifically Expressed in Serotonin Neurons in the Transgenic Mouse Brain

(A) Tph2-IR neurons are located in the DRN (Alexa Fluor 594, red). YFP-IR neurons, indicating ChR2(C128S) expression, are also observed in the DRN (Alexa Fluor 488, green). The merged picture shows the specific expression of ChR2(C128S) in the serotonin neurons in the DRN in transgenic mice (top row). Scale bar, 100 μm. YFP-IR is observed in the soma and dendrites. Aside from the serotonin neurons, no other expression of ChR2(C128S) was observed in the DRN. The bottom row presents higher magnifications of the square region in the top row. Scale bar, 50 μm.

(B) Blue light sensitivity in the activity of ChR2-expressing serotonin neurons in the presence of tetrodotoxin. Under whole-cell current clamp mode, blue light induced depolarization in a light intensity-dependent manner. Light intensities are 0.01 mW, 0.3 mW, 1.3 mW, and 2.5 mW (from left to right).

(C) Bar graph summarizing the data in (B) (n = 7). *p < 0.05 compared with blue light responses of ChR2(−) serotonin neurons.

(D) In whole-cell current clamp mode, blue light illumination (500 ms pulses, blue line) induced instantaneous depolarization and increased spontaneous action potentials in ChR2-expressing serotonin neurons. Yellow light illumination (500 ms pulse, yellow line) stopped spontaneous action potentials.

(E) Average firing rate (n = 10) during a 5 s period. Pre, from 5 s before blue light onset to blue light onset; blue, from blue light onset to yellow light onset; post-1, from yellow light onset to 5 s afterward; post-2, from 5 s after yellow light onset to 10 s after yellow light onset. **p < 0.01, ***p < 0.001 compared with pre.

(F) Average firing rate (n = 10) during a 5 s period. Pre, from 5 s before yellow light (500 ms pulse) onset; post, from yellow light onset to 5 s after yellow light onset.

(G) Example of serotonin neural activity after 5 s yellow light stimulation.

(H) Average firing rates (n = 10) of serotonin neurons after a 5 s yellow light stimulation.

(I) Changes in serotonin efflux in the medial prefrontal cortex (mPFC) after blue and yellow light stimulation of DRN serotonin neurons were detected by microdialysis.

(J) Serotonin efflux by continuous yellow (CY) light (n = 7), 1 s of transient blue (TB) light (n = 6), and continuous blue (CB) light (n = 8).

*p < 0.05, ***p < 0.001 compared with baseline. NS, not significant. All error bars represent the SEM.

(conditioned reinforcer) at a tone site and then to wait for delayed food (primary reward) at a reward site (Figure 2A; see the Supplemental Experimental Procedures). In experiment 1, we examined whether light stimulation itself can affect animals' behaviors (Figures 2A and S2A). A tone wait error ratio (the number of tone wait errors/the number of total tone site nose pokes) in the yellow light trials (0.67 ± 0.03, n = 13 tests) was not significantly different from that in the no light trials (0.66 ± 0.04, n = 13 tests; t(12) = 0.21, p = 0.84, paired t test) (Figure 2C). In experiment 2, to test the effect of DRN serotonin neuron stimulation, we applied the blue or yellow light during the tone delay period randomly in half of the trials each (Figures 2B and S2B). We observed that the tone wait error ratio during the blue light trials (0.55 ± 0.02, n = 45 tests) was reduced significantly compared with that in the yellow light trials (0.73 ± 0.02, n = 45 tests; t(44) = 9.42, $p = 4.10 \times 10^{-12}$, paired t test) (Figure 2D; see Figures S4A–S4E and the Supplemental Results for individual analyses of each mouse). We confirmed, in three wild-type mice, that the tone wait error ratio in the blue light trials was not significantly different from that in the yellow light trials (t(38) = 0.27, p = 0.79, n = 39 tests, paired t test) (Figure 2E).

We next examined how the blue light stimulation modulated waiting behavior with various tone delay durations of 0.6–1.5 s (experiment 3; Figure S2C). As the tone delay period increased, the number of tone wait errors increased in both the yellow and blue light trials, but the increase was less steep in the blue light trials ($F(1,196) = 16.03$, $p = 8.86 \times 10^{-5}$, analysis of covariance) (Figure 2F). The ratios of the tone wait errors in the blue light trials to those in the yellow light trials in the longer delay lengths (1.2 and 1.5 s) were significantly smaller than the ratio for the 0.6 s delay length (F(3,72) = 3.91, p = 0.012,

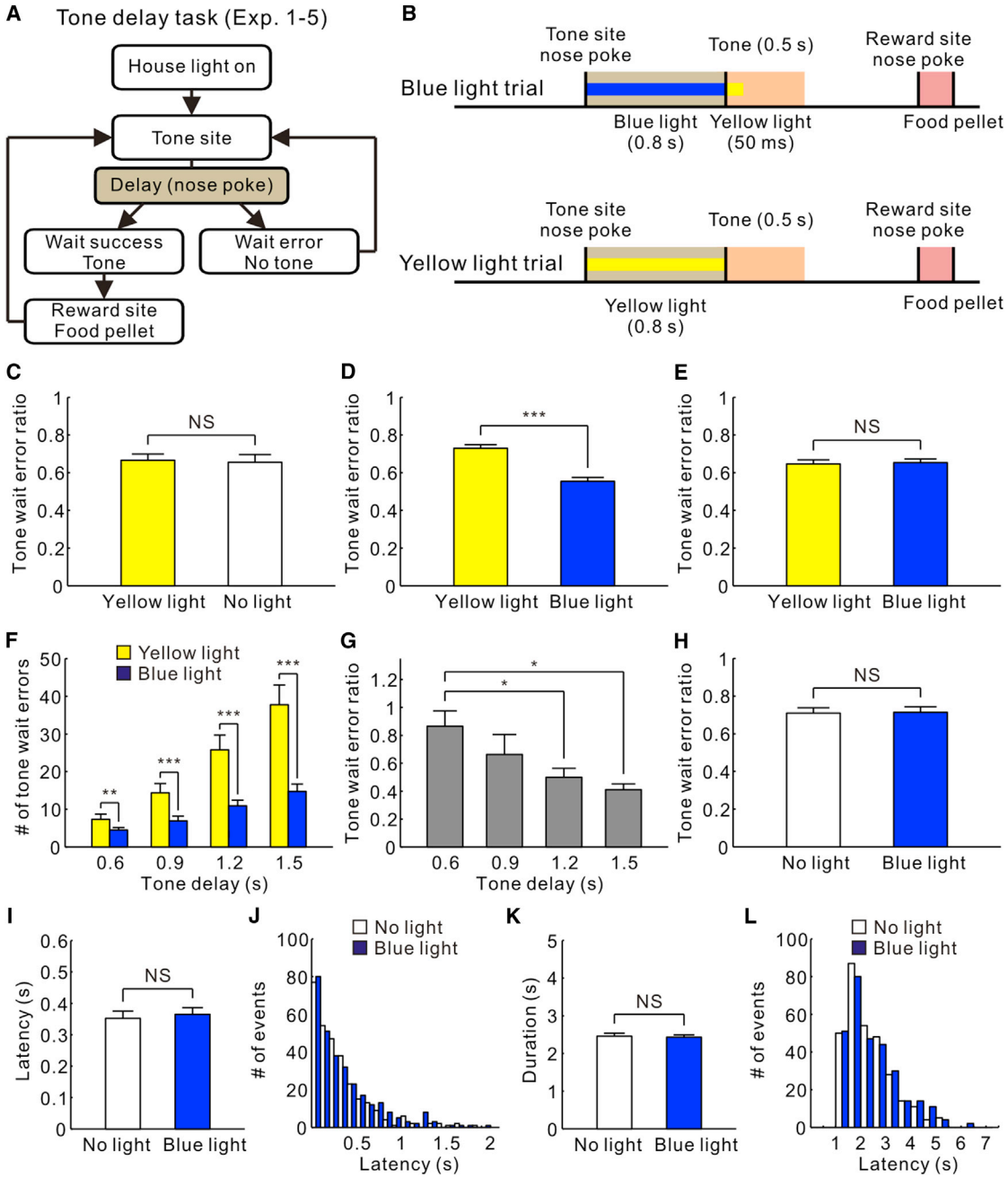

Figure 2. Blue Light Stimulation in the DRN Reduces the Tone Wait Error Ratio

(A) Diagram of the test in which optogenetic stimulation was applied during the tone delay period (experiments 1–5).

(B) Time sequence of blue light trials and yellow light trials applied during the tone delay period (experiment 2). Blue light stimulation was followed by a brief yellow light stimulation (50 ms). Blue and yellow bars denote blue and yellow light stimulations, respectively. Brown and red regions denote tone and reward delay periods, respectively. Orange regions denote the duration of the tone presentation.

(C) Tone wait error ratios in the yellow light trial (yellow bar) and the no light trial (white bar) are shown (experiment 1) (n = 12 tests with 3 mice).

(D) Tone wait error ratios in the yellow light trial (yellow bar) and the blue light trial (blue bar) (experiment 2) (n = 45 tests with 5 mice).

(E) Tone wait error ratios in the yellow light trial (yellow bar) and the blue light trial (blue bar) in wild-type mice (experiment 10) (n = 39 tests with 3 mice).

(F) Number of tone wait errors in the extended tone delay test in which the tone delay (0.6, 0.9, 1.2, and 1.5 s) was increased gradually every 10 trials (experiment 3) (n = 25 tests with 4 mice).

(G) Ratio between the number of tone wait errors in the blue light trials and in the yellow light trials during experiment 3.

(H) Tone wait error ratios in the no light trial (white bar) and the blue light trial (blue bar) in which the blue light was applied offset to the tone delay period (experiment 4) (n = 34 tests with 5 mice).

(I) Mean latencies in the no blue light trial and the blue light trial in which the blue light (0.8 s) was applied at the same time as the tone presentation (experiment 5) (n = 300 trials, each trial with 5 mice).

(J) Distribution of the latencies in the no blue light and the blue light trials.

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one-way repeated measures ANOVA; p = 0.029 for 0.6 versus 1.2 s delay, p = 0.010 for 0.6 versus 1.5 s delay, post hoc Bonferroni test) (Figure 2G). These results show that the precisely timed activation of DRN serotonin neurons enables the animals to wait for a delayed reinforcer and that the effect is more pronounced when more patience is required.

We further investigated whether the timing of the blue light stimulation is critical in reducing the tone wait errors by applying the blue light stimulation before the animals started nose-poking at the tone site (experiment 4; Figure S2D). In this case, the tone wait error ratio during the blue light trials did not significantly change compared with the tone wait error ratio during the no blue light trials (t(33) = 0.054, p = 0.96, n = 34 tests, paired t test) (Figure 2H). This result indicates that the precise timing of the serotonin neuron activation is critical for promoting the animals' decision to be patient.

Reduced Tone Wait Errors Are Not due to Behavioral Inhibition

To test whether the reduction of tone wait errors via blue light stimulation is due to an inhibition of motor behavior, we applied the blue light at the time of tone presentation in half of the trials and measured the response latency until the exit from the nose poke hole (experiment 5; Figure S2E). There was no significant difference in the latency between the blue light trials (0.37 ± 0.02 s, n = 300) and the no blue light trials (0.35 ± 0.02 s, n = 300; t(598) = 0.41, p = 0.69, two-tailed t test) (Figures 2I and 2J). We also examined whether the subjects' ongoing behavior was inhibited by serotonin neural activation by applying 0.8 s of blue light 1 s after the onset of the tone in half of the trials (experiment 13; Figure S2F). There was no significant difference in the time to nose poke onset to the reward site between the blue light (2.43 ± 0.06 s, n = 300) and no blue light trials (2.46 ± 0.08 s, n = 300; t(598) = 0.26, p = 0.79, two-tailed t test) (Figures 2K and 2L). Finally, we examined whether serotonin neural activation outside of the task induces stereotype behaviors (freezing, grooming, and rearing) (experiment 14). We found no remarkable behavioral responses (see the Supplemental Results for blue light stimulation during free moving). These results show that optogenetic stimulation of the serotonin neurons does not cause a significant inhibitory effect on the motor behavior of moving out of a nose poke hole. Therefore, it is unlikely that the reduced tone wait errors were simply due to motor inhibition (see the Supplemental Discussion for serotonin and behavioral inhibition).

Serotonin Neuron Stimulation Prolongs Waiting Time

To quantify how serotonin neuron activation affects the animals' patience in waiting for a delayed reward, we designed a random reward delay (RRD) test in which the reward delay was randomly set to 3, 6, or 9 s or to infinity (meaning reward omission trials), whereas the tone delay was fixed at 0.2 s (Figure 3A). To also verify the effectiveness of transient blue light stimulation on the sustained activation of the serotonin neurons via ChR2(C128S), we performed the following two experiments: a continuous blue light versus a continuous yellow light experiment (experiment 6; Figures 3B and S3A) and a 0.8 s transient blue light versus no light experiment (experiment 7; Figure S3B). In both experiments, with the yellow or no light stimulation, the number of reward wait errors increased significantly in 9 s delay trials compared with the number of reward wait errors in 3 s delay trials (p < 0.014, paired t test). The number of reward wait errors in 9 s delay trials was reduced significantly in both the continuous and the 0.8 s transient blue light stimulations (t(23) = 3.68, p = 0.0012, n = 24 tests, continuous blue light versus continuous yellow light; t(20) = 3.87, p = 9.5×10^{-4}, n = 21 tests, 0.8 s transient blue light versus no light; paired t test) (Figures 3C and 3F).

In the omission trials, the wait duration in the continuous blue light trials (17.45 ± 0.49 s, n = 119 trials) was significantly longer than the wait duration in the continuous yellow light trials (11.96 ± 0.37 s, n = 119 trials) (t(236) = 8.71, p = 5.4×10^{-16}, two-tailed t test) (Figures 3D and 3E; see Figures S4F–S4I and the Supplemental Results for individual analyses of each mouse). For wild-type mice (n = 3), we confirmed that the wait duration in the continuous blue light trials (12.51 ± 0.27 s, n = 138 trials) was not significantly different from that in the continuous yellow light trials (12.74 ± 0.28 s, n = 138 trials; t(274) = 0.58, p = 0.56, two-tailed t test). In experiment 7, the wait duration in the 0.8 s transient blue light trials (16.9 ± 0.41 s, n = 105 trials) was significantly longer than the wait duration in the no light trials (12.54 ± 0.4 s, n = 105 trials) (t(208) = 7.82, p = 2.73×10^{-13}, two-tailed t test) (Figures 3G and 3H). These results provide direct evidence that the activation of serotonin neurons causes prolonged waiting for a delayed reward.

Based on our previous observation that putative serotonin neural activity in rats dropped immediately before the animals gave up waiting [19], we hypothesized that artificial serotonin neural activation is effective specifically when an animal makes a decision to either keep waiting for an expected reward or to abandon it. To test this hypothesis, we compared the wait durations in omission trials by activating the serotonin neurons in either the first 10 s (experiment 8) or 10 s (experiment 9) after beginning the wait using 0.8 s of transient blue light stimulation (Figures S3C and S3D). When the serotonin neuron activation was stopped after 10 s by 50 ms of transient yellow light, the mice stopped waiting after 4.1 ± 0.23 s (n = 99 trials) (Figures 3I and 3J). The wait duration was significantly shorter than that with a yellow light stimulation after the end of waiting (16.68 ± 0.27 s, n = 99 trials; t(196) = 6.22, p = 2.90×10^{-9}; two-tailed t test). When 0.8 s of transient blue light was applied after 10 s of waiting, the wait duration (16.61 ± 0.28 s, n = 114 trials) was comparable with that with 0.8 s of transient blue light at the beginning of the wait (16.57 ± 0.27 s, n = 114 trials; t(226) = 0.25, p = 0.80; two-tailed t test) (Figures 3K and 3L). These results support our hypothesis that serotonin neural activation facilitates patience for a future reward specifically when the animal is engaged in deciding whether to keep waiting (see the Supplemental Discussion for serotonin and impulsivity).

Prolonged Waiting Is Not due to the Rewarding Effects of Serotonin Stimulation

We examined whether the decrease in waiting errors and the prolonged waiting time during omission trials could be because serotonin neural activation increased the value of

(K) Mean durations between tone onset and nose poke onset to the reward site in the no light trial and the blue light trial in which the blue light (0.8 s) was applied 1 s after the onset of the tone presentation (experiment 13) (n = 300 trials, each trial with 3 mice).
(L) Distribution of the durations in the no light and the blue light trials.
*p < 0.05, **p < 0.01, ***p < 0.001. NS, not significant. Error bars represent the SEM. See also Figures S1, S2, and S4.

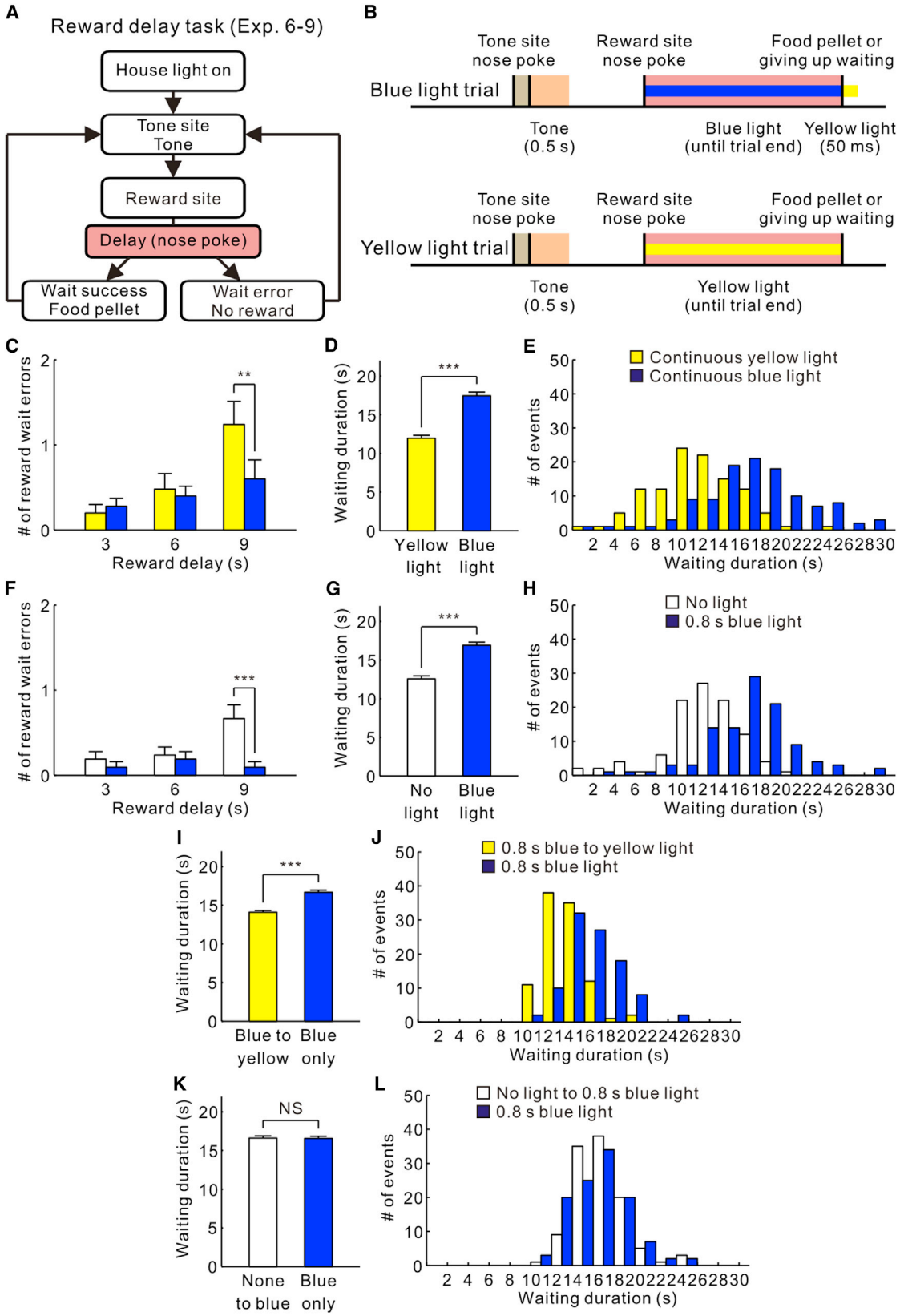

Figure 3. Blue Light Stimulation in the DRN Prolongs the Duration of Waiting for a Delayed Reward

(A) Diagram of the test in which optogenetic stimulation was applied during the reward delay period (experiments 6–9).
(B) Time sequence of the blue light trial and the yellow light trial applied during the reward delay period (experiment 6).
(C) Number of reward wait errors during the random reward delay in experiment 6 (n = 25 tests with 4 mice).

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the food reward (reward value test, experiment 11). For this experiment, three transgenic mice were trained to perform a simple RRD (sRRD) test in which the reward delay was randomly set to 2, 3, or 4 s or to infinity (Figure 4A). With 0.8 s of blue light stimulation at the onset of the nose poke to the reward site, the wait duration in the omission trials (8.26 ± 0.14 s, n = 120 trials) was significantly longer than that with no light stimulation (7.08 ± 0.12 s, n = 120 trials; $t(236) = 6.58$, $p = 3.04 \times 10^{-10}$, two-tailed t test) (Figures 4B and 4C). This confirmed the effectiveness of blue light stimulation in these mice. We then compared the animals' performance under three conditions: one pellet condition, one pellet + blue light condition, and two pellets condition (Figure 4D). In the two pellets condition, the wait duration during omission trials (8.88 ± 0.16 s, n = 145) was significantly more prolonged than in the one pellet condition (7.06 ± 0.10 s, n = 165; $t(308) = 9.89$, $p = 3.24 \times 10^{-20}$; for each mouse, $p < 2.03 \times 10^{-5}$; two-tailed t test) (Figures 4E and 4F). This shows that an increase in reward value could enhance waiting behavior for a delayed reward. However, under the one pellet + blue light condition, in which 0.8 s of blue light was applied at the same time as the delivery of one food pellet (Figure 4D), the wait duration during omission trials (7.10 ± 0.08 s, n = 125) was not significantly different from than under the one pellet condition ($t(268) = 0.31$, $p = 0.76$; for each mouse, $p > 0.30$; two-tailed t test) (Figures 4E and 4F). These results suggest that, with our light stimulation protocol, serotonin neural activation during food consumption did not increase the food value and did not affect the waiting duration for delayed reward.

There are reports that electrical self-stimulation of the DRN and optogenetic stimulation of DRN Pet-1-positive neurons induce a rewarding effect [26, 27]. To examine the possibility that the prolonged wait duration during the omission trial with optogenetic stimulation (experiments 6–9) was due to the reinforcing effect of serotonin neuron activation, we used the spontaneous nose pokes to the reward site of our mice during the intertask interval (defined as a rest period). If the optogenetic activation of DRN serotonin neurons has a reinforcing effect, the mice should prolong their nose-poking to receive more serotonin stimulation. To test this hypothesis, we randomly activated serotonin neurons in 50% of the spontaneous nose pokes to the reward site during the rest period (reward effect test, experiment 12) (Figure 4G). There was no significant difference between the nose poke duration with (1.55 ± 0.07 s, n = 350 times) or without (1.53 ± 0.07 s, n = 350 times) serotonin neural activation ($t(698) = 0.22$, $p = 0.83$; for each mouse, $p > 0.65$; two-tailed t test) (Figures 4H and 4I). Therefore, it is difficult to explain the prolonged wait duration with optogenetic stimulation through its reinforcing effects (see the Supplemental Discussion for serotonin and rewarding effect). This result also confirms that optogenetic activation during nose pokes did not inhibit motor responses that cause prolonged nose poking.

Discussion

We optogenetically activated DRN serotonin neurons while the mice performed a sequential tone-food waiting task in which we manipulated the length of delay to the conditioned reinforcer tone and to the food reward. We found that serotonin neuron activation while the mice waited for the tone significantly reduced tone wait errors. When the duration of the tone delay was increased, the reduction of tone-wait errors became more prominent with the longer tone delays. Furthermore, we found that serotonin neural activation did not affect the reaction time to exit from the tone site or the latency to arrive at the reward site, which means that the reduced wait errors were not due to behavioral inhibition. We also found that, when serotonin neurons were activated during the variable delay periods for a food reward (3, 6, or 9 s or infinity), the number of reward wait errors was reduced significantly in the 9 s wait trials. In the reward omission trials, the waiting time of the mice was significantly longer in the serotonin activation trials compared with the trials with no activation. With optogenetic stimulation in the early and late phases of waiting, we observed that serotonin neuron activation was effective only when it was given during the time when the animals usually quit waiting. These results establish, for the first time, the causality in which the precisely timed activation of serotonin neurons modulates animals' waiting behavior to receive a delayed reward. These results and our previous studies [18, 19, 21] demonstrate that the activation of DRN serotonin neurons enhances patience for a future reward when the animal is deciding whether to keep waiting or to abandon the wait (see the Supplemental Discussion for serotonin and patience).

A recent study demonstrated that the optogenetic activation of prefrontal cortex neurons projecting to the DRN increased effortful behavioral responses to challenging situations [28]. However, the net effect of the prefrontal input to the DRN serotonin neurons remains unclear because of the possible activation of inhibitory interneurons. The combination of neural recording with optogenetic and pharmacological manipulation will enable us to dissect the afferent input, local circuit, and cellular autoregulatory mechanisms that shape the activities of serotonin neurons [29]. This approach should also enable us to reveal the brain's algorithm for the regulation of patience [17].

Supplemental Information

Supplemental Information includes Supplemental Results, Supplemental Discussion, Supplemental Experimental Procedures, and four figures and

(D) Mean duration of waiting during the omission trials under continuous blue light versus continuous yellow light conditions (experiment 6) (n = 125 trials with 4 mice).
(E) Distribution of the wait durations during the omission trials in the continuous blue light and the continuous yellow light trials in experiment 6.
(F) Number of reward wait errors during the random reward delay in experiment 7 (n = 21 tests with 4 mice).
(G) Mean duration of waiting during the omission trials under transient blue light versus no light conditions (experiment 7) (n = 105 trials with 4 mice).
(H) Distribution of the wait durations in (G).
(I) Mean duration of waiting during the omission trials under transient blue light versus transient blue to yellow light conditions (experiment 8) (n = 99 trials with 4 mice).
(J) Distribution of the wait durations in experiment 8.
(K) Mean duration of waiting during the omission trials under transient blue light versus no light to transient blue light conditions (experiment 9) (n = 114 trials with 4 mice).
(L) Distribution of the wait durations in experiment 9.
**p < 0.01, ***p < 0.001. NS, not significant. Error bars represent the SEM. See also Figures S1, S3, and S4.

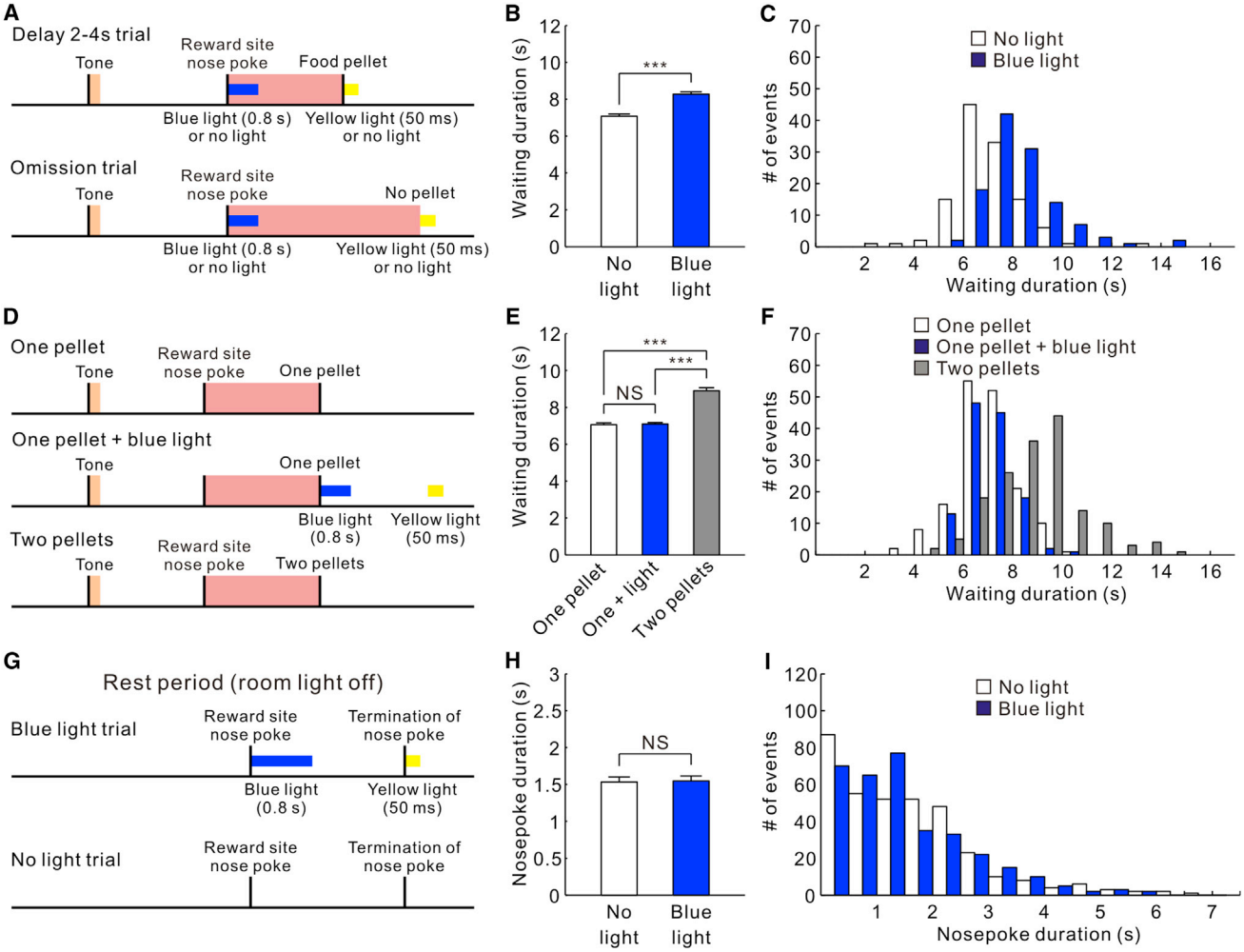

Figure 4. Blue Light Stimulation in the DRN Does Not Induce a Reinforcing Effect or Change the Reward Value to Prolong Wait Duration

(A) Time sequence of the sRRD test in which the reward delay was chosen randomly from 2, 3, or 4 s or infinity (experiment 11).
(B) Mean duration of waiting during the omission trials in the no light and the blue light trials (n = 120 trials with 3 mice).
(C) Distribution of the wait durations during the omission trials in the no light and the blue light trials.
(D) Time sequence of three reward conditions in the sRRD test: one pellet, one pellet + blue light, and two pellets (experiment 11).
(E) Mean duration of waiting during the omission trials under one pellet (n = 165), one pellet + blue light (n = 125), and two pellets (n = 145) conditions.
(F) Distribution of the wait durations during the omission trials under the three reward conditions.
(G) Time sequence of the reward effect test in which serotonin neurons were activated during half of the spontaneous nose pokes to the reward site (experiment 12).
(H) Mean duration of nose pokes in the no light (n = 350) and the blue light (n = 350) trials.
(I) Distribution of nose poke durations during the reward effect test in the no light and the blue light trials.
***$p < 0.001$. NS, not significant. Error bars represent the SEM.

can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.07.041.

Author Contributions

K.W.M., K.M., and K.D. designed the research. K.W.M. and K.M. performed the experiments and analyzed the data. K.M., K.W.M., and K.D. discussed the results and wrote the manuscript. K.F.T., A.Y., and A.T. generated the Tph2-tTA::tetO-ChR2(C128S)-EYFP knockin mice. A.Y. and S.T. performed the immunohistochemistry and in vitro electrophysiological recordings. All authors edited the manuscript.

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